EXHIBIT A

1: EMBO J. 1986 Dec 1;5(12):3361-6. Related Articles, Lin

DNA sequences required for expression of a Dictyosteliu actin gene.

Cohen SM, Knecht D, Lodish HF, Loomis WF.

A 2.8-kb fragment of 5' non-coding DNA from the Dictyostelium actin 15 gene has previously been shown to contain all of the cis-acting DN sequence elements required for normal developmentally-regulated transcription of actin gene fusion RNAs when reintroduced into the genome by DNA-mediated transformation. Deletion analysis of this promoter fragment indicates that all of the necessary information is contained within a 270-bp fragment of actin 15 5' non-coding DNA. This fragment contains four short G/C-rich repeated sequences that are also found in other co-regulated Dictyostelium actin genes. A 12-b consensus sequence, AAAAATGGGG/ATT, is present in the regions essential for expression of two different Dictyostelium actin genes, actin 6 and actin 15, but is absent from an actin gene showing a different temporal pattern of developmental regulation. Deletion analysis and DNase I footprinting implicate this sequence as a functional cis-acting element required for transcription of the actin 15 gene.

PMID: 3028782 [PubMed - indexed for MEDLINE]

EXHIBIT B

1: Mol Gen Genet. 1990 Sep;223(3):401-6.

cis regulatory elements directing tuber-specific and sucrose-inducible expression of a chimeric class I patatir promoter/GUS-gene fusion.

Liu XJ, Prat S, Willmitzer L, Frommer WB.

Institut fur Genbiologische Forschung Berlin GmbH, FRG.

The 5'-upstream region of the class I patatin gene B33 directs strong expression of the beta-glucuronidase (GUS) reporter gene in potato tubers and in leaves treated with sucrose. Cis-acting elements affecting specificity and level of expression were identified by deletion analysis in transgenic potato plants. A putative tuber-specific element is located downstream from position -195. Nuclear proteins present in leaf and tuber extracts bind specifically to a conserved AT rich motif within this region. A DNA fragment between -183 and -143, including the binding site is, however, not able to enhance the expression of a truncated 35S promoter from cauliflower mosaic virus. Independent positive elements contributing to a 100-fold increase relative to the basic tuber-specific element are located between -228 and -195; -736 and -509, -930 and -736 and -1512 and -951. Sucrose inducibility is controlled by sequences downstream of position -228, indicating that the tuber-specific and sucrose-inducible elements are in close proximity.

PMID: 2270080 [PubMed - indexed for MEDLINE]

EXHIBIT C

1: Proc Natl Acad Sci U S A. 1990 Jan;87(2):603-7. Related Articles, Lin

Wound-inducible nuclear protein binds DNA fragments that regulate a proteinase inhibitor II gene from potato.

Palm CJ, Costa MA, An G, Ryan CA.

Institute of Biological Chemistry, Washington State University, Pullma 99164-6340.

Deletion analysis from the 3' to the 5' end of the promoter region of th wound-inducible potato proteinase inhibitor IIK gene has identified a 421-base sequence at -136 to -557 that is necessary for expression. Utilizing DNA band-shift assays, a 10-base sequence within the 421-base region was found to bind a nuclear protein from wounded tomatc leaves. This 10-base sequence is adjacent to an 8-base consensus sequence at -147 to -155 that is present in the promoter region of several elicitor-inducible genes from various other plants. The evidenc suggests that a complex set of cis- and trans-acting elements within the -136 to -165 region of the potato IIK gene may be involved with th signaling mechanisms that regulate the inducibility of this gene in response to pest and pathogen attacks.

PMID: 2405385 [PubMed - indexed for MEDLINE]

EXHIBIT D

1: Nucleic Acids Res. 1991 Apr 11;19(7):1571-6.

Upstream sequences of rice proliferating cell nuclear antigen (PCNA) gene mediate expression of PCNA-GUS chimeric gene in meristems of transgenic tobacco plants.

Kosugi S, Suzuka I, Ohashi Y, Murakami T, Arai Y.

Institute of Applied Biochemistry, University of Tsukuba, Ibaraki, Japa

The transgenic tobacco plants have been generated that express the E. coli beta-glucuronidase (GUS) gene under control of the promoter from the rice proliferating cell nuclear antigen (PCNA, DNA polymeras auxiliary protein) gene. GUS expression detected in situ by staining with the chromogenic substrate, 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (X-Gluc), was restricted to meristems in the organs of the transgenic tobacco plants. This expression responded to the phytohormones which promote callus formation. Furthermore, in situ thymidine uptake showed that the GUS expression pattern corresponded well to the active sites of DNA synthesis. Deletion analysis of the 5' upstream sequence confined the GUS expression pattern to a fragment extending 263 bp upstream of the transcription start site of the rice PCNA gene. Thus, we have identified this fragmei as a main regulatory element of the rice PCNA gene promoter.

PMID: 1709277 [PubMed - indexed for MEDLINE]

EXHIBIT E

1: Plant Physiol. 1994 May;105(1):357-67.

Identification of a light-responsive region of the nuclear gene encoding the B subunit of chloroplast glyceraldehyde 3-phosphate dehydrogenase from Arabidopsis thaliana.

Kwon HB, Park SC, Peng HP, Goodman HM, Dewdney J, Shih MC

Department of Biological Sciences, University of Iowa, Iowa City 52242.

We report here the identification of a cis-acting region involved in light regulation of the nuclear gene (GapB) encoding the B subunit of chloroplast glyceraldehyde 3-phosphate dehydrogenase from Arabidopsis thaliana. Our results show that a 664-bp GapB promoter fragment is sufficient to confer light induction and organ-specific expression of the Escherichia coli beta-glucuronidase reporter gene (Gus) in transgenic tobacco (Nicotiana tabacum) plants. Deletion analysis indicates that the -261 to -173 upstream region of the GapB gene is essential for light induction. This region contains four direct repeats with the consensus sequence 5'-ATGAA(A/G)A-3' (Gap boxes). Deletion of all four repeats abolishes light induction completel In addition, we have linked a 109-bp (-263 to -152) GapB upstream fragment containing the four direct repeats in two orientations to the -92 to +6 upstream sequence of the cauliflower mosaic virus 35S basa promoter. The resulting chimeric promoters are able to confer light induction and to enhance leaf-specific expression of the Gus reporter gene in transgenic tobacco plants. Based on these results we concluc that Gap boxes are essential for light regulation and organ-specific expression of the GapB gene in A. thaliana. Using gel mobility shift assays we have also identified a nuclear factor from tobacco that interacts with GapA and GapB DNA fragments containing these Gap boxes. Competition assays indicate that Gap boxes are the binding sites for this factor. Although this binding activity is present in nuclear extracts from leaves and roots of light-grown or dark-treated tobacco plants, the activity is less abundant in nuclear extracts prepared from leaves of dark-treated plants or from roots of greenhouse-grown plant In addition, our data show that this binding factor is distinct from the GT-1 factor, which binds to Box II and Box III within the light-

United States Patent **6,177,613**
Coughlan , et al. **January 23, 2001**

Seed-preferred ***promoter***

Abstract

The present invention provides a composition and method for regulating expression of heterologous nucleotide sequences in a plant. The composition is a novel nucleic acid sequence for a seed-preferred promoter. A method for expressing a heterologous nucleotide sequence in a plant using the promoter sequence is also provided. The method comprises transforming a plant cell to contain a heterologous nucleotide sequence operably linked to the seed-preferred promoter of the present invention and regenerating a stably transformed plant from the transformed plant cell.

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Assignee: **Pioneer Hi-Bred International, Inc.** (Des Moines, IA)
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Intern'l Class: C12N 005/04; C12N 015/82; C12N 015/90; A01H 005/00; A01H 005/10

Fi ld of Search: 435/69.1,320.1,410,412,414,415,416,419,468 536/23.6,24.1 800/278,281,287,295,298,312,314,317.3,320,320.1,320.2,320.3,322

References Cited [Referenced By]

Other Refer nces

Kim et al, Plant Mol. Biol., vol. 24, pp. 105-117, 1994.
Grossi et al, Plant Sci., vol. 103, pp. 189-198, 1994.
Kodama et al, Plant Physiol., vol. 107, pp. 1177-1185, 1995.

Marcellino et al, FEBS Lett., vol. 385, pp. 154-158, 1996.
Fromm et al, Bio/Technol., vol. 8, pp. 833-839, 1990.
Fromm et al., "Inheritance and Expression of Chimeric Genes in the Progeny of Transgenic Maize Plants", Bio/Technology, 8:833-839 (1990).
Grossi de Sa et al., "Functional studies on a seed-specific promoter from Brazil nut 2S gene", Plant Science, 103:189-198 (1994).
Kim et al., "A 20 nucleotide upstream element is essential for the nopaline synthase (nos) promoter activity", Plant Molecular Biology, 24:105-117 (1994).
Kodama et al., "Fatty Acid Desaturation during Chilling Acclimation is One of the Factors Involved in Conferring Low-Temperature Tolerance to Young Tobacco Leaves", Plant Physiol., 107:117-1185 (1995).
Marcellino et al., "Modified 2S albumins with improved tryptophan content are correctly expressed in transgenic tobacco plants", FEBS Letters, 385:154-158 (1996).

Primary Examiner: Hutzell; Paula K.
Assistant Examiner: Mehta; Ashwin D.
Attorney, Agent or Firm: Pioneer Hi-Bred International, Inc.

Claims

That which is claimed:

1. An isolated promoter that comprises nucleotide sequences having at least 65% sequence ***identity*** to SEQ ID NO: 1 wherein the % sequence ***identity*** is based on the entire sequence and is determined by BLAST analysis under default parameters and said isolated promoter has the transcription initiating properties of SEQ ID NO: 1.

2. An isolated promoter that comprises a nucleotide sequence as set forth in SEQ ID NO: 1.

3. An expression cassette comprising a promoter and a nucleotide sequence of interest operably linked to the promoter, wherein the promoter comprises the nucleotide sequence set forth in SEQ ID NO: 1.

4. An expression cassette comprising a promoter and a nucleotide sequence of interest operably linked to the promoter, wherein the promoter has at least 65% sequence ***identity*** to SEQ ID NO: 1, wherein the % sequence ***identity*** is based on the entire sequence and is determined by BLAST analysis under default parameters and said promoter has the transcription initiating properties of SEQ ID NO: 1.

5. A transformation vector comprising the expression cassette of claim 4.

6. A plant cell tranformed with the expression cassette of claim 4.

7. A plant stably transformed with the expression cassette of claim 4.

8. A method for selectively expressing a nucleotide sequence of interest in a plant seed, the method comprising:

responsive element of the RbcS-3A gene of pea.

PMID: 8029358 [PubMed - indexed for MEDLINE]
